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**Structures of Enzymes in the Alginate Biosynthetic Pathway of *Pseudomonas aeruginosa*.**

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**Introduction:** Cystic Fibrosis (CF) patients are prone to secondary infections in the lungs. One of the infectious organisms is *Pseudomonas aeruginosa*. In the CF lung this organism transforms into a mucoid state that overproduces an alginate coating – a polysaccharide. In this state *P. aeruginosa* is resistant to antibiotics and the innate immune response of the lung. Various proteins are involved in the synthesis of alginate (Shanker et al, 1995) and expression is highly controlled. These proteins have not been structurally characterized and the understanding of their mechanisms would be greatly enhanced by the structural knowledge obtained.

**Methods and Materials:** In this study we have obtained large single crystals of two native, recombinant proteins involved in the alginate pathway for X-ray diffraction studies. These are the Phosphomannomutase –PMM (Regni, 2000) and GDP-mannose dehydrogenase – GMD (310Å unit cell) proteins. Seleno-methionine substituted protein crystals or heavy-atom soaked crystals will be used for multiple-wavelength anomalous dispersion (MAD) phasing experiments. All data collection has been carried out at 100-110K.

**Results:** We have obtained a 1.44Å native data set of a PMM active site mutant, S108A. The structure has now been solved for the native protein and the S108A mutant. Data sets of PMM potentially complexed with its various cofactors and substrates have been obtained to near completeness with maximal resolutions of between 18.Å to 2.1Å. A putative GMD starting complex data set has been collected to 2.25Å but with a twinning factor of 0.084.

**Conclusions:** We have been able to progress rapidly with the structure solution of PMM and are beginning to investigate the putative complex and mutant data sets. The GMD is progressing slowly due to the need for detectors with a 2-theta arm to collect high-resolution data and merohedral twinning complicating the phasing.

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**References:** S. Shanker, R. W. Ye, D. Schlichtman and A.M. Chakrabarty. "Exopolysaccharide Alginate Synthesis in *Pseudomonas aeruginosa*: Enzymology and Regulation of Gene Expression." Advances in Enzymology and Related Areas of Molecular Biology, 70, 221, 1995.

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